

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
21 February 2002 (21.02.2002)

PCT

(10) International Publication Number  
**WO 02/13851 A1**

(51) International Patent Classification<sup>7</sup>: **A61K 38/17**

(21) International Application Number: **PCT/US00/22777**

(22) International Filing Date: 17 August 2000 (17.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant (for all designated States except US): **THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West 7th Street, Austin, TX 78701 (US).**

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BOLDOGH, Istvan [HU/US]; 302 Holiday Drive, #17, Galveston, TX 77550 (US). STANTON, John, G. [US/US]; 3026 112th Street N., Texas City, TX 77591 (US). HUGHES, Thomas, K., Jr. [US/US]; Route 1, Box 225 B-1, Galveston, TX 77554 (US).**

(74) Agent: **MUETING, Ann, M.; Muetting, Raasch & Gebhardi, P.A., P.O. Box 581415, Minneapolis, MN 55458-1415 (US).**

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

**WO 02/13851 A1**

(54) Title: USE OF COLOSTRININ, CONSTITUENT PEPTIDES THEREOF, AND ANALOGS THEREOF TO PROMOTE NEURAL CELL DIFFERENTIATION

(57) Abstract: The present invention discloses a use of colostrinin, a constituent peptide thereof, and/or an analog thereof as a neural cell regulator in animals including humans.

**USE OF COLOSTRININ, CONSTITUENT PEPTIDES THEREOF, AND  
ANALOGS THEREOF TO PROMOTE NEURAL CELL  
DIFFERENTIATION**

5

**Background of the Invention**

Colostrum is a component of the milk of mammals during the first few days after birth. Colostrum is a thick yellowish fluid and is the first lacteal secretion post parturition and contains a high concentration of immunoglobulins (IgG, IgM, and IgA) and a variety of non-specific proteins. Colostrum also contains various cells such as granular and stromal cells, neutrophils, monocyte/macrophages, and lymphocytes. Colostrum also includes growth factors, hormones, and cytokines. Unlike mature breast milk, colostrum contains low sugar, low iron, but is rich in lipids, proteins, mineral salts, vitamins, and immunoglobulins.

Colostrum also includes or contains a proline-rich polypeptide aggregate or complex, which is referred to as colostrinin. One peptide fragment of colostrinin is Val-Glu-Ser-Tyr-Val-Pro-Leu-Phe-Pro (SEQ ID NO:31), which is disclosed in International Publication No. WO-A-98/14473. Colostrinin and this fragment have been identified as useful in the treatment of disorders of the central nervous system, neurological disorders, mental disorders, dementia, neurodegenerative diseases, Alzheimer's disease, motor neurone disease, psychosis, neurosis, chronic disorders of the immune system, diseases with a bacterial and viral aetiology, and acquired immunological deficiencies as set forth in International Publication No. WO-A-98/14473.

Although certain uses for colostrinin have been identified, it would represent an advancement in the art to discover and disclose other uses for colostrinin, or a component thereof, that are not readily ascertainable from the information currently known about colostrinin or its constituents.

**Summary of the Invention**

The present invention relates to the use of colostrinin, at least one constituent (i.e., component) peptide thereof, at least one active analog thereof (e.g., peptide having an N-terminal sequence equivalent to an N-terminal sequence of at least one of the colostrinin constituent peptides), and combinations thereof, as promoters of neural cell differentiation. These agents can be used *in vitro* or *in vivo*, including internal use in patients, particularly animals (including mammals such as humans).

10        In one embodiment, the present invention provides a method for promoting cell differentiation. The method includes contacting cells (preferably, pluripotent cells) with a neural cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to change the cells in morphology to form neural cells (i.e., nerve-like cells). The cells can be present in a cell culture, an organ, a tissue, or an organism. Preferably, the cells are mammalian cells, and more preferably, human cells. The neural cell regulator is preferably a constituent peptide of colostrinin, such as those described herein (SEQ ID NOs:1-34).

15        The present invention also provides a method for promoting neural cell differentiation in a patient (preferably, a human). The method includes administering to the patient a neural cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to promote differentiation (i.e., a change in morphology) of cells to form neural cells (i.e., nerve-like cells).

20        The present invention further provides a method for treating damaged (which typically possess less than complete function and may be completely nonfunctional) neural cells. The method includes contacting damaged neural cells with a neural cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to convert the damaged neural cells to functional neural cells. This method can occur *in vitro* or *in vivo*. An *in vivo* method for treating damaged (e.g., nonfunctional) neural cells in a patient includes administering to

the patient a neural cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to convert damaged neural cells to functional neural cells.

In other embodiments, the invention provides the use of a neural cell regulator in the manufacture of a medicament for use in the methods described herein.

As used herein, "neural" and "nerve-like" are used interchangeably. Such cells have morphologies resembling nerve cells. For example, a central body with neurite outgrowth. As used herein, nonfunctional neural cells are 10 those that do not transmit information by, e.g., acetylcholine, but morphologically resemble nerve cells, and functional neural cells are those that do transmit information using mediators such as acetylcholine and morphologically resemble nerve cells.

As used herein, "a" or "an" means one or more, such that combinations 15 of active agents (i.e., active immunological regulators or blood cell differentiation promoters), for example, can be used in the compositions and methods of the invention. Thus, a composition that includes "a" polypeptide refers to a composition that includes one or more polypeptides.

"Amino acid" is used herein to refer to a chemical compound with the 20 general formula:  $\text{NH}_2\text{---CRH---COOH}$ , where R, the side chain, is H or an organic group. Where R is organic, R can vary and is either polar or nonpolar (i.e., hydrophobic). The amino acids of this invention can be naturally occurring or synthetic (often referred to as nonproteinogenic). As used herein, an organic group is a hydrocarbon group that is classified as an aliphatic group, a cyclic 25 group or combination of aliphatic and cyclic groups. The term "aliphatic group" means a saturated or unsaturated linear or branched hydrocarbon group. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example. The term "cyclic group" means a closed ring hydrocarbon group that is classified as an alicyclic group, aromatic group, or heterocyclic group. The term "alicyclic" 30 group" means a cyclic hydrocarbon group having properties resembling those of aliphatic groups. The term "aromatic group" refers to mono- or polycyclic

aromatic hydrocarbon groups. As used herein, an organic group can be substituted or unsubstituted.

The terms "polypeptide" and "peptide" are used interchangeably herein to refer to a polymer of amino acids. These terms do not connote a specific length of a polymer of amino acids. Thus, for example, the terms oligopeptide, protein, and enzyme are included within the definition of polypeptide or peptide, whether produced using recombinant techniques, chemical or enzymatic synthesis, or naturally occurring. This term also includes polypeptides that have been modified or derivatized, such as by glycosylation, acetylation, phosphorylation, and the like.

The following abbreviations are used throughout the application:

A = Ala = Alanine	T = Thr = Threonine
V = Val = Valine	C = Cys = Cysteine
L = Leu = Leucine	Y = Tyr = Tyrosine
15 I = Ile = Isoleucine	N = Asn = Asparagine
P = Pro = Proline	Q = Gln = Glutamine
F = Phe = Phenylalanine	D = Asp = Aspartic Acid
W = Trp = Tryptophan	E = Glu = Glutamic Acid
M = Met = Methionine	K = Lys = Lysine
20 G = Gly = Glycine	R = Arg = Arginine
S = Ser = Serine	H = His = Histidine

#### Brief Description of the Drawings

**Figure 1. Effect of NGF and constituent peptides of colostrinin on morphology of PC12 cells.** 3 x 10<sup>4</sup> cells per well were seeded in 24 x well plates and 24 hours (h) later cells were treated with NGF or colostrinin, or its constituent peptides, as described in the Examples Section. Six days after treatment, cells were fixed in formaldehyde and stained to visualize morphological changes of cells. Mock-treated cells (A), NGF-treated cells (B).

25 Lower panel demonstrate typical morphological changes of PC12 cells after exposure to SEQ ID NO:1 (C) or SEQ ID NO:2 (D, D1).

30

### Detailed Description of Preferred Embodiments

The inventors have found that colostrinin, at least one constituent peptide thereof, and/or at least one active analog thereof (e.g., a peptide having an N-terminal sequence equivalent to an N-terminal sequence of at least one of the 5 colostrinin constituent peptides) can be used as neural cell regulators. Such regulators promote the differentiation of cells (e.g., pluripotent cells) such that there is a change in morphology to form neural cells (which can be present in tissues and organs such as brain or ganglion). This can occur *in vitro* or *in vivo*, including internally in animals (including mammals such as humans). These 10 regulators can also convert damaged (e.g., nonfunctional) neural cells to functional neural cells.

Such neural cell regulators are referred to herein as "active agents." Significantly, such agents can be administered alone or in various combinations to a patient (e.g., animals including humans) as a medication or dietary (e.g., 15 nutrient) supplement in a dose sufficient to cause nerve cell increase throughout the patient's body, in a specific tissue site, or in a collection of tissues (organs).

The differentiation process of cells in the nervous system is regulated by the action of differentiation and growth factors including NGF. For example, NGF binding to its receptor tyrosine kinase, TrkA, initiates various molecular 20 interactions including tyrosine phosphorylation of proteins and the action of the Ras/Raf/MEK/MAPK pathway (Chao et al., Cell, 68, 995-997 (1992); and Marshall et al., Cell, 80, 179-185 (1995)). NGF induces the production of reactive nitric oxide (NO), and NO is required for NGF-induced cytostasis and differentiation (Peunova et al., Nature, 375, 68-73 (1995)), suggesting that free 25 radical molecules may exert a regulatory role in certain types of cellular differentiation.

It is important to promote nerve cell differentiation (i.e., promote differentiation of cells to form neural cells) and/or conversion of damaged nerve cells where there has been significant damage to nerve cells that can occur in a 30 wide variety of situations. The active agents described herein can be used individually, in various combinations, or combined with other previously known or newly invented pharmacological agents. The promotion of nerve cell

differentiation responses can be taken advantage of, for example, in cell, tissue, or organ regeneration, repair, and replacement.

In preferred embodiments, the present invention provides methods for promoting neural cell differentiation (i.e., differentiation of cells to form neural cells) and converting nonfunctional neural cells to functional neural cells.

5 Whether it be *in vivo* or *in vitro*, these methods involve monitoring the level of increase in functional nerve cells and/or changes in the morphology of cells formed using phenotypic markers as disclosed by Fillmore et al., J. Neurosci. Res., 31, 662-669 (1992) and Levi et al., Mol. Neurobiol., 2, 201-226 (1988).

10 Specific *in vitro* methods are described in the Examples Section.

Colostrinin is composed of peptides, the aggregate of which has a molecular weight range between about 5.8 to about 26 kiloDaltons (kDa) determined by polyacrylamide gel electrophoresis. It has a greater concentration of proline than any other amino acid. Ovine colostrinin has been found to have a 15 molecular weight of about 18 kDa and includes three non-covalently linked subunits having a molecular weight of about 6 kDa and has about 22 wt-% proline. Ovine colostrinin has also been shown to contain the following number of residues per subunit: lysine - 2; histidine - 1; arginine - 0; aspartic acid - 2; threonine - 4; serine - 3; glutamic acid - 6; proline - 11; glycine - 2; alanine - 0; 20 valine - 5; methionine - 2; isoleucine - 2; leucine - 6; tyrosine - 1; phenylalanine - 3; and cysteine - 0.

Colostrinin has been found to include a number of peptides ranging from 3 amino acids to 22 amino acids or more. These can be obtained by various known techniques, including isolation and purification involving electrophoresis 25 and synthetic techniques. The specific method of obtaining colostrinin and SEQ ID NO:31 is described in International Publication No. WO-A-98/14473. Using HPLC and Edelman Degradation, over 30 constituent peptides of colostrinin have been identified, which can be classified into several groups: (A) those of unknown precursor; (B) those having a  $\beta$ -casein homologue precursor; (C) those 30 having a  $\beta$ -casein precursor; and (D) those having an annexin precursor. These peptides are described in International Patent Application PCT/GB00/02128, filed June 2, 2000, claiming priority to June 2, 1999, and can be synthesized

according to the general method described in the Examples Section. These peptides (i.e., constituent peptides of colostrinin), which can be derived from colostrinin or chemically synthesized, include: MQPPPLP (SEQ ID NO:1); LQTPQPLLQVMMEPQGD (SEQ ID NO:2); DQPPDVEKPDLQPFQVQS 5 (SEQ ID NO:3); LFFFLPVNVNLP (SEQ ID NO:4); DLEMPVLPVEPFPFV (SEQ ID NO:5); MPQNFYKLPQM (SEQ ID NO:6); VLEMKFPPPPQETVT (SEQ ID NO:7); LKPFPKLKVEVFPFP (SEQ ID NO:8); VVMEV (SEQ ID NO:9); SEQP (SEQ ID NO:10); DKE (SEQ ID NO:11); FPPPK (SEQ ID NO:12); DSQPPV (SEQ ID NO:13); DPPPPQS (SEQ ID NO:14); SEEMP 10 (SEQ ID NO:15); KYKLQPE (SEQ ID NO:16); VLPPNVG (SEQ ID NO:17); VYPFTGPIPN (SEQ ID NO:18); SLPQNILPL (SEQ ID NO:19); TQTPVVVPPF (SEQ ID NO:20); LQPEIMGVPKVKETMVPK (SEQ ID NO:21); HKEMPFPKYPVEPFTESQ (SEQ ID NO:22); SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23); SWMHQPP (SEQ ID NO:24); 15 QPLPPTVMFP (SEQ ID NO:25); PQSVLS (SEQ ID NO:26); LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27); AFLLYQE (SEQ ID NO:28); RGPFPILV (SEQ ID NO:29); ATFNRYQDDHGEELKSL (SEQ ID NO:30); VESYVPLFP (SEQ ID NO:31); FLLYQEPVLGPVR (SEQ ID NO:32); LNF (SEQ ID NO:33); and MHQPPQPLPPTVMFP (SEQ ID NO:34). 20 These can be classified as follows: (A) those of unknown precursor include SEQ ID NOs:2, 6, 7, 8, 10, 11, 14, and 33; (B) those having a  $\beta$ -casein homologue precursor include SEQ ID NOs:1, 3, 4, 5, 9, 12, 13, 15, 16, 17, and 31; (C) those having a  $\beta$ -casein precursor include SEQ ID NOs:18 (casein amino acids 74-83), 19 (casein amino acids 84-92), 20 (casein amino acids 93-102), 21 25 (casein amino acids 103-120), 22 (casein amino acids 121-138), 23 (casein amino acids 139-156), 24 (casein amino acids 157-163), 25 (casein amino acids 164-173), 26 (casein amino acids 174-179), 27 (casein amino acids 180-201), 28 (casein amino acids 202-208), 29 (casein amino acids 214-222), 32 (casein amino acids 203-214), and 34 (casein amino acids 159-173); and (D) those 30 having an annexin precursor include SEQ ID NO:30 (annexin amino acids 203-220).

A preferred group of such peptides includes: MQPPPLP (SEQ ID NO:1); LQTPQPLLQVMMMEPQGD (SEQ ID NO:2); DQPPDVEKPDLQPFQVQS (SEQ ID NO:3); LFFFPLVVNVLP (SEQ ID NO:4); DLEMPVLPVEPFPFV (SEQ ID NO:5); MPQNFYKLPQM (SEQ ID NO:6); VLEMKFPPPPQETVT (SEQ ID NO:7); LKPFPKLKVEVFPFP (SEQ ID NO:8); and combinations thereof.

The polypeptides of SEQ ID NOs:1-34 can be in their free acid form or they can be amidated at the C-terminal carboxylate group. The present invention also includes analogs of the polypeptides of SEQ ID NOs:1-34, which 10 includes polypeptides having structural similarity with SEQ ID NOs:1-34. These peptides can also form a part of a larger peptide. An "analog" of a polypeptide includes at least a portion of the polypeptide, wherein the portion contains deletions or additions of one or more contiguous or noncontiguous amino acids, or containing one or more amino acid substitutions. An "analog" 15 can thus include additional amino acids at one or both of the terminii of the polypeptides listed above. Substitutes for an amino acid in the polypeptides of the invention are preferably conservative substitutions, which are selected from other members of the class to which the amino acid belongs. For example, it is well-known in the art of protein biochemistry that an amino acid belonging to a 20 grouping of amino acids having a particular size or characteristic (such as charge, hydrophobicity and hydrophilicity) can generally be substituted for another amino acid without substantially altering the structure of a polypeptide.

For the purposes of this invention, conservative amino acid substitutions are defined to result from exchange of amino acids residues from within one of 25 the following classes of residues: Class I: Ala, Gly, Ser, Thr, and Pro (representing small aliphatic side chains and hydroxyl group side chains); Class II: Cys, Ser, Thr and Tyr (representing side chains including an -OH or -SH group); Class III: Glu, Asp, Asn and Gln (carboxyl group containing side chains); Class IV: His, Arg and Lys (representing basic side chains); Class V: 30 Ile, Val, Leu, Phe and Met (representing hydrophobic side chains); and Class VI: Phe, Trp, Tyr and His (representing aromatic side chains). The classes also include related amino acids such as 3Hyp and 4Hyp in Class I; homocysteine in

Class II; 2-amino adipic acid, 2-aminopimelic acid,  $\gamma$ -carboxyglutamic acid,  $\beta$ -carboxy aspartic acid, and the corresponding amino acid amides in Class III; ornithine, homoarginine, N-methyl lysine, dimethyl lysine, trimethyl lysine, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, homoarginine, sarcosine and 5 hydroxylysine in Class IV; substituted phenylalanines, norleucine, norvaline, 2-amino octanoic acid, 2-aminoheptanoic acid, statine and  $\beta$ -valine in Class V; and naphthylalanines, substituted phenylalanines, tetrahydroisoquinoline-3-carboxylic acid, and halogenated tyrosines in Class VI.

Preferably, the active analogs of colostrinin and its constituent peptides 10 include polypeptides having a relatively large number of proline residues. Because proline is not a common amino acid, a "large number" preferably means that a polypeptide includes at least about 15% proline (by number), and more preferably at least about 20% proline (by number). Most preferably, active analogs include more proline residues than any other amino acid.

15 As stated above, active analogs of colostrinin and its constituent peptides include polypeptides having structural similarity. Structural similarity is generally determined by aligning the residues of the two amino acid sequences to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the 20 alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. Preferably, two amino acid sequences are compared using the Blastp program, version 2.0.9, of the BLAST 2 search algorithm, available at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. Preferably, the default values for all 25 BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x\_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, structural similarity is referred to as "identity." Preferably, an active analog of colostrinin or its constituent peptides 30 has a structural similarity to colostrinin or one or more of its constituent peptides (preferably, one of SEQ ID NOs:1-34) of at least about 70% identity, more

preferably, at least about 80% identity, and most preferably, at least about 90% identity.

Colostrinin or any combination of its peptide components or active analogs thereof can be derived (preferably, isolated and purified) naturally such

- 5 as by extraction from colostrum or can be synthetically constructed using known peptide polymerization techniques. For example, the peptides of the invention may be synthesized by the solid phase method using standard methods based on either t-butyloxycarbonyl (BOC) or 9-fluorenylmethoxy-carbonyl (FMOC) protecting groups. This methodology is described by G.B. Fields et al. in
- 10 Synthetic Peptides: A User's Guide, W.M. Freeman & Company, New York, NY, pp. 77-183 (1992). Moreover, gene sequence encoding the colostrinin peptides or analogs thereof can be constructed by known techniques such as expression vectors or plasmids and transfected into suitable microorganisms that will express the DNA sequences thus preparing the peptide for later extraction
- 15 from the medium in which the microorganism are grown. For example, U.S. Patent No. 5,595,887 describes methods of forming a variety of relatively small peptides through expression of a recombinant gene construct coding for a fusion protein which includes a binding protein and one or more copies of the desired target peptide. After expression, the fusion protein is isolated and cleaved using
- 20 chemical and/or enzymatic methods to produce the desired target peptide.

The peptides used in the methods of the present invention may be employed in a monovalent state (i.e., free peptide or a single peptide fragment coupled to a carrier molecule). The peptides may also be employed as conjugates having more than one (same or different) peptide fragment bound to a single carrier molecule. The carrier may be a biological carrier molecule (e.g., a glycosaminoglycan, a proteoglycan, albumin or the like) or a synthetic polymer (e.g., a polyalkyleneglycol or a synthetic chromatography support). Typically, ovalbumin, human serum albumin, other proteins, polyethylene glycol, or the like are employed as the carrier. Such modifications may increase the apparent affinity and/or change the stability of a peptide. The number of peptide fragments associated with or bound to each carrier can vary, but from

about 4 to 8 peptides per carrier molecule are typically obtained under standard coupling conditions.

For instance, peptide/carrier molecule conjugates may be prepared by treating a mixture of peptides and carrier molecules with a coupling agent, such 5 as a carbodiimide. The coupling agent may activate a carboxyl group on either the peptide or the carrier molecule so that the carboxyl group can react with a nucleophile (e.g., an amino or hydroxyl group) on the other member of the peptide/carrier molecule, resulting in the covalent linkage of the peptide and the carrier molecule. For example, conjugates of a peptide coupled to ovalbumin 10 may be prepared by dissolving equal amounts of lyophilized peptide and ovalbumin in a small volume of water. In a second tube, 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride (EDC; ten times the amount of peptide) is dissolved in a small amount of water. The EDC solution was added to the peptide/ovalbumin mixture and allowed to react for a number of 15 hours. The mixture may then dialyzed (e.g., into phosphate buffered saline) to obtain a purified solution of peptide/ovalbumin conjugate. Peptide/carrier molecule conjugates prepared by this method typically contain about 4 to 5 peptides per ovalbumin molecule.

The present invention also provides a composition that includes one or 20 more active agents (i.e., colostrinin, at least one constituent peptide thereof, or active analog thereof) of the invention and one or more carriers, preferably a pharmaceutically acceptable carrier. The methods of the invention include administering to, or applying to the skin of, a patient, preferably a mammal, and more preferably a human, a composition of the invention in an amount effective 25 to produce the desired effect. The active agents of the present invention are formulated for enteral administration (oral, rectal, *etc.*) or parenteral administration (injection, internal pump, *etc.*). The administration can be via direct injection into tissue, interarterial injection, interventional injection, or other internal administration procedures, such as through the use of an implanted 30 pump, or via contacting the composition with a mucus membrane in a carrier designed to facilitate transmission of the composition across the mucus membrane such as a suppository, eye drops, inhaler, or other similar

administration method or via oral administration in the form of a syrup, a liquid, a pill, capsule, gel coated tablet, or other similar oral administration method. The active agents can be incorporated into an adhesive plaster, a patch, a gum, and the like, or it can be encapsulated or incorporated into a bio-erodible matrix 5 for controlled release.

The carriers for internal administration can be any carriers commonly used to facilitate the internal administration of compositions such as plasma, sterile saline solution, IV solutions or the like. Carriers for administration through mucus membranes can be any well-known in the art. Carriers for 10 administration oral can be any carrier well-known in the art.

The formulations may be conveniently presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the 15 formulations are prepared by uniformly and intimately bringing the active agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulations.

Formulations suitable for parenteral administration conveniently include a sterile aqueous preparation of the active agent, or dispersions of sterile 20 powders of the active agent, which are preferably isotonic with the blood of the recipient. Isotonic agents that can be included in the liquid preparation include sugars, buffers, and sodium chloride. Solutions of the active agent can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions of the active agent can be prepared in water, ethanol, a polyol (such as glycerol, 25 propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, glycerol esters, and mixtures thereof. The ultimate dosage form is sterile, fluid, and stable under the conditions of manufacture and storage. The necessary fluidity can be achieved, for example, by using liposomes, by employing the appropriate particle size in the case of dispersions, or by using surfactants. 30 Sterilization of a liquid preparation can be achieved by any convenient method that preserves the bioactivity of the active agent, preferably by filter sterilization. Preferred methods for preparing powders include vacuum drying and freeze

drying of the sterile injectible solutions. Subsequent microbial contamination can be prevented using various antimicrobial agents, for example, antibacterial, antiviral and antifungal agents including parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. Absorption of the active agents over a prolonged 5 period can be achieved by including agents for delaying, for example, aluminum monostearate and gelatin.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as tablets, troches, capsules, lozenges, wafers, or cachets, each containing a predetermined amount of the active agent 10 as a powder or granules, as liposomes containing the active agent, or as a solution or suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion, or a draught. The amount of active agent is such that the dosage level will be effective to produce the desired result in the subject.

Nasal spray formulations include purified aqueous solutions of the active 15 agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids. Ophthalmic formulations are prepared by 20 a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye. Topical formulations include the active agent dissolved or suspended in one or more media such as mineral oil, DMSO, polyhydroxy alcohols, or other bases used for topical pharmaceutical formulations.

25 Useful dosages of the active agents can be determined by comparing their *in vitro* activity and the *in vivo* activity in animal models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art; for example, see U.S. Patent No. 4,938,949.

The tablets, troches, pills, capsules, and the like may also contain one or 30 more of the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as

magnesium stearate; a sweetening agent such as sucrose, fructose, lactose or aspartame; and a natural or artificial flavoring agent. When the unit dosage form is a capsule, it may further contain a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or

5 to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac, or sugar and the like. A syrup or elixir may contain one or more of a sweetening agent, a preservative such as methyl- or propylparaben, an agent to retard crystallization of the sugar, an agent to increase the solubility of any other

10 ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol, a dye, and flavoring agent. The material used in preparing any unit dosage form is substantially nontoxic in the amounts employed. The active agent may be incorporated into sustained-release preparations and devices.

15

### Examples

The invention will be further described by reference to the following detailed examples. The examples are meant to provide illustration and should not be construed as limiting the scope of the present invention.

## 20 MATERIALS AND METHODS

### **Preparation of Peptides:**

1. Wash pre-loaded resin with DMF (dimethylformamide), then drain completely.
2. Add 10 ml of 20% piperidine/DMF to resin. Shake for 5 minutes, then

25 drain.

3. Add another 10 ml of 20% piperidine/DMF. Shake for 30 minutes.
4. Drain reaction vessel and wash resin with DMF four times. Then wash once with DCM (dichloromethanol). Check beads using the ninhydrin test - the beads should be blue.
- 30 5. The coupling step was carried out as follows:
  - a. Prepare the following solution: 1 mmole Fmoc (i.e. fluorenylmethyloxycarbonyl) amino acid 2.1 ml of 0.45 M HBTU/HOBt (1

mmol) (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/N-hydroxybenzotriazole-H<sub>2</sub>O) 348 µl of DIEA (2 mmol) (diisopropylethylamine); and

- 5        b.        Add the solution to the resin and shake for a minimum of 30 minutes.
6.        Drain reaction vessel and wash the resin again with DMF four times and with DCM once.
7.        Perform the ninhydrin test: If positive (no colour) - proceed to step 2 and continue synthesis; If negative (blue colour) - return to step 5 and recouple the 10 same Fmoc amino acid.
8.        After the synthesis was complete, the peptide was cleaved from the resin with 5% H<sub>2</sub>O, 5% phenol, 3% Thionisole, 3% EDT (ethanedithiol), 3% triisopropylsilane and 81% TFA for 2 hours.
9.        After 2 hours, filter into cold MTBE (methyl t-butyl ether). The 15 precipitated peptide was then washed twice with cold MTBE and dried under nitrogen gas.
10.       The molecular weight of the synthesised peptides was checked by Matrix-Assisted Laser Desorption Time-of-Flight Mass Spectroscopy (LDMS), and the purity was checked by HPLC using a C-18, 300 Angstrom, 5 µm 20 column.

Cells: PC12 cell line derived from medullary pheochromocytoma cells were used to undertake studies described below. PC12 cells were obtained from the American Type Culture Collection and maintained in RPMI-1640 25 supplemented with 10% fetal bovine serum (HYCLONE Inc), penicillin (100 U/ml) and streptomycin (100 µg/ml).

Methods: To evaluate the effect of colostrum, colostrinin and its component peptides on cell differentiation 3 x 10<sup>4</sup> logarithmically replicating 30 (70% confluence) PC12 cells were seeded in 24 x well plates and cells were allowed to adhere and grow for 24 hours. Serum containing media were aspirated and replaced for serum-free RPMI containing appropriate amount of

antibiotics. In four parallel, increasing concentrations (0.1, 1, 10 and 100 µg per ml) of colostrum, colostrinin and its component peptides were added directly into the media and incubated at 37°C. As a positive control, nerve growth factor (NGF) 7S (Gibco-BRL) was used at 100 ng per ml concentration (Chao et al., 5 *Cell*, **68**, 995-997 (1992); and Marshall et al., *Cell*, **80**, 179-185 (1995)). Phorbol 12-myristate 13-acetate (TPA: 10 ng per ml) was used as a negative control. Eight hours later the media were changed and RPMI-1640 was added containing 1% or 10% fetal bovine serum. The cultures were microscopically investigated 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> days after treatment. Six days after treatment cells 10 were fixed with paraformaldehyde (4%) and stained with 0.01% crystal violet solution. Excess of dyes were removed by ethanol washing. The final evaluation took place using a microscopy (*Axiophot2* Zeiss Inc., Germany).

## RESULTS

15 Control non-treated cells demonstrated the usual rounded morphology, continued to replicate and reach 70% to 80% confluency during 7 days experimental period. The mock-treated control and TPA exposed cells showed a low background level of differentiation (less than 0.01%). In the assay system used herein, NGF (100 ng per ml) mediated a cell cycle arrest as previously described (Chao et al., *Cell*, **68**, 995-997 (1992)). In several experiments, in the 20 presence of 10% fetal bovine serum NGF-mediated an induction of cell differentiation that observed in 45±11% of cells. When cells were subjected to NGF-mediated differentiation in the presence of 1% serum 5% to 10% of cells showed morphological changes. The differentiated cells showed typical neuron- 25 like morphology. In parallel experiments, nine component peptides, colostrinin and colostrum were tested. The results are summarized in Table 1.

In the presence of 1% fetal bovine serum there was no cell differentiation observed. These data indicate that some of the serum factor(s) are required to 30 biological effect of these compounds. On the other hand, in the presence of 10% serum, the component peptides, colostrinin as well as colostrum have induced cell differentiation in PC12 cells. The morphological changes (fibroblast-like, epitheloid, neuron-like) are shown in Figure 1. These data are in agreement with

cytokine inducing activity of these peptides. For example, IFN-gamma and nerve growth factor was shown to induce similar signal transduction cascades (Peunova et al., Nature, 375, 68-73 (1995)).

**Table 1.** Effect of colostrum, colostrinin, and its component peptides on morphology (differentiation) of medullary pheochromocytoma (PC12) cells.

	Peptide	Concentration μg/ml	Cell morphology		
			1% FBS	10% FBS	
5	SEQ ID NO:1	100	-	+/-	
		10	-	++	epitheloid
		1.0	-	+	neuron-like
		0.1	-	+/-	
	SEQ ID NO:7	100	-	+/-	
		10	-	+	fibroblast-like
		1.0	-	+	neuron-like
		0.1	-	-	
10	SEQ ID NO:8	100	-	-	
		10	-	+	fibroblast-like
		1.0	-	++	neuron-like
		0.1	-	-	
	SEQ ID NO:3	100	-	+	
		10	-	+	fibroblast-like
		1.0	-	+/-	neuron-like
		0.1	-	-	
10	SEQ ID NO:2	100	-	+	
		10	+	++	fibroblast-like
		1.0	-	++	neuron-like
		0.1	-	+/-	
	SEQ ID NO:4	100	-	++	fibroblast-like
		10	-	++	epitheloid
		1.0	-	+	neuron-like
		0.1	-	-	
10	SEQ ID NO:5	100	-	+	fibroblast-like
		10	-	+/-	epitheloid
		1.0	-	-	neuron-like
		0.1	-	-	
	SEQ ID NO:6*	100	-	+	
		10	-	+	fibroblast-like
		1.0	-	+/-	epitheloid
		0.1	-	-	

SEQ ID NO:31	100	NT	NT	
	10	-	+/-	
	1.0	-		
	0.1	-		
Colostrinin	100	NT	NT	
	10	+	++	
	1.0	-	++	
	0.1	-	-	
Colostrum	100	NT	NT	
	10	-	++	
	1.0	-	++	
	0.1	-	+/-	
NGF	0.1	++++	++++	neuron-like
TPA (Negative Control)	0.1			rounded
Control	-	-	-	rounded

NT = Not Tested; - =  $\leq 0.01\%$  (back-ground); + = 1%; ++ = 1-5%; +++ = 6-15%; +++++ =

10 >15%

\* Although the cells treated with SEQ ID NO:6 did not show neuron-like cell morphology upon visual inspection, additional tests are needed to definitively prove such morphology changes did not occur.

15 Although the invention has been disclosed with reference to its preferred embodiments, from reading this description those of skill in the art may appreciate changes and modification that may be made which do not depart from the scope and spirit of the invention as described above and claimed hereafter. All references, patents, and patent applications cited herein are incorporated  
20 herein by reference in their entirety as if individually incorporated.

#### **Sequence Listing Free Text**

The following are all synthetic peptide sequences.

SEQ ID NO:1 MQPPPLP

25 SEQ ID NO:2 LQTPQPLLQVMMEPQGD

SEQ ID NO:3 DQPPDVEKPDQLQPFQVQS

SEQ ID NO:4 LFFFPLPVNVLP

SEQ ID NO:5 DLEMPVLPVEPFPFV

	SEQ ID NO:6	MPQNFYKLPQM
	SEQ ID NO:7	VLEMKFPPPPQETVT
	SEQ ID NO:8	LKPFPKLKVEVFPFP
	SEQ ID NO:9	VVMEV
5	SEQ ID NO:10	SEQP
	SEQ ID NO:11	DKE
	SEQ ID NO:12	FPPPK
	SEQ ID NO:13	DSQPPV
	SEQ ID NO:14	DPPPQS
10	SEQ ID NO:15	SEEMP
	SEQ ID NO:16	KYKLQPE
	SEQ ID NO:17	VLPPNVG
	SEQ ID NO:18	VYPFTGPIPN
	SEQ ID NO:19	SLPQNILPL
15	SEQ ID NO:20	TQTPVVVPPF
	SEQ ID NO:21	LQPEIMGVPKVKETMVPK
	SEQ ID NO:22	HKEMPFPKYPVEPFTESQ
	SEQ ID NO:23	SLTLTDVEKLHLPLPLVQ
	SEQ ID NO:24	SWMHQPP
20	SEQ ID NO:25	QPLPPTVMFP
	SEQ ID NO:26	PQSMLS
	SEQ ID NO:27	LSQPKVLPVPQKAVPQRDMPIQ
	SEQ ID NO:28	AFLLYQE
	SEQ ID NO:29	RGPFPILV
25	SEQ ID NO:30	ATFNRYQDDHGEEILKSL
	SEQ ID NO:31	VESYVPLFP
	SEQ ID NO:32	FLLYQEPVLGPVR
	SEQ ID NO:33	LNF
	SEQ ID NO:34	MHQPPQPLPPTVMFP

**We claim:**

1. A method for promoting cell differentiation, the method comprising contacting cells with a neural cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to change the cells in morphology to form neural cells.
2. The method of claim 1 wherein the cells are present in a cell culture, an organ, a tissue, or an organism.
3. The method of claim 1 wherein the cells are mammalian cells.
4. The method of claim 3 wherein the cells are human cells.
5. The method of claim 1 wherein the cells are pluripotent cells.
6. The method of claim 1 wherein the neural cell regulator is a constituent peptide of colostrinin.
7. The method of claim 6 wherein the neural cell regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDQLQPFQVQS (SEQ ID NO:3), LFFFPLPVVNVL (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), VVMEV (SEQ ID NO:9), SEQP (SEQ ID NO:10), DKE (SEQ ID NO:11), FPPPK (SEQ ID NO:12), DSQPPV (SEQ ID NO:13), DPPPPQS (SEQ ID NO:14), SEEMP (SEQ ID NO:15), KYKLQPE (SEQ ID NO:16), VLPPNVG (SEQ ID NO:17), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), LQPEIMGVPKVKETMVPK (SEQ ID NO:21), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22), SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23), SWMHQPP (SEQ ID NO:24), QPLPPTVMFP (SEQ ID NO:25), PQSVLS (SEQ ID NO:26),

LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27), AFLLYQE (SEQ ID NO:28), RGPFPLV (SEQ ID NO:29), ATFNRYQDDHGEELKSL (SEQ ID NO:30), VESYVPLFP (SEQ ID NO:31), FLLYQEPVLGPVR (SEQ ID NO:32), LNF (SEQ ID NO:33), and MHQPPQPLPPTVMFP (SEQ ID NO:34), and combinations thereof.

8. The method of claim 7 wherein the neural cell regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDQLQPFQVQS (SEQ ID NO:3), LFFFPLVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), and combinations thereof.

9. A method for promoting neural cell differentiation in a patient, the method comprising administering to the patient a neural cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to promote differentiation of cells to form neural cells.

10. The method of claim 9 wherein the patient is a human.

11. The method of claim 9 wherein the neural cell regulator is a constituent peptide of colostrinin.

12. The method of claim 11 wherein the neural cell regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDQLQPFQVQS (SEQ ID NO:3), LFFFPLVVNVLP (SEQ ID NO:4), DLEMPVLPVEPFPFV (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), VVMEV (SEQ ID NO:9), SEQP (SEQ ID NO:10), DKE (SEQ ID NO:11), FPPPK (SEQ ID NO:12), DSQPPV (SEQ ID NO:13), DPPPPQS (SEQ ID NO:14), SEEMP (SEQ ID NO:15), KYKLQPE (SEQ ID NO:16), VLPPNVG

(SEQ ID NO:17), VYPFTGPIPN (SEQ ID NO:18), SLPQNILPL (SEQ ID NO:19), TQTPVVVPPF (SEQ ID NO:20), LQPEIMGVPKVKETMVPK (SEQ ID NO:21), HKEMPFPKYPVEPFTESQ (SEQ ID NO:22),  
SLTLTDVEKLHLPLPLVQ (SEQ ID NO:23), SWMHQPP (SEQ ID NO:24),  
QPLPPTVMFP (SEQ ID NO:25), PQSVLS (SEQ ID NO:26),  
LSQPKVLPVPQKAVPQRDMPIQ (SEQ ID NO:27), AFLLYQE (SEQ ID NO:28), RGPFPILV (SEQ ID NO:29), ATFNRYQDDHGEEILKSL (SEQ ID NO:30), VESYVPLFP (SEQ ID NO:31), FLLYQEPVLGPVR (SEQ ID NO:32),  
LNF (SEQ ID NO:33), and MHQPPQPLPPTVMFP (SEQ ID NO:34), and  
combinations thereof.

13. The method of claim 12 wherein the neural cell regulator is selected from the group of MQPPPLP (SEQ ID NO:1), LQTPQPLLQVMMEPQGD (SEQ ID NO:2), DQPPDVEKPDQLQPFQVQS (SEQ ID NO:3), LFFFLPVVNVL (SEQ ID NO:4), DLEMPVLPVEPFVF (SEQ ID NO:5), MPQNFYKLPQM (SEQ ID NO:6), VLEMKFPPPPQETVT (SEQ ID NO:7), LKPFPKLKVEVFPFP (SEQ ID NO:8), and combinations thereof.

14. A method for treating damaged neural cells, the method comprising contacting nonfunctional neural cells with a neural cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to convert the damaged neural cells to functional neural cells.

15. A method for treating damaged neural cells in a patient, the method comprising administering to the patient a neural cell regulator selected from the group of colostrinin, a constituent peptide thereof, an analog thereof, and combinations thereof, under conditions effective to convert damaged neural cells to functional neural cells.

A: Control  
B: NGF (100 ng/ml)  
C: SEQ ID NO:1 (1 micro g/ml)  
D: SEQ ID NO:2 (1 micro g/ml)  
D1: SEQ ID NO:2 (0.1 micro g/ml)

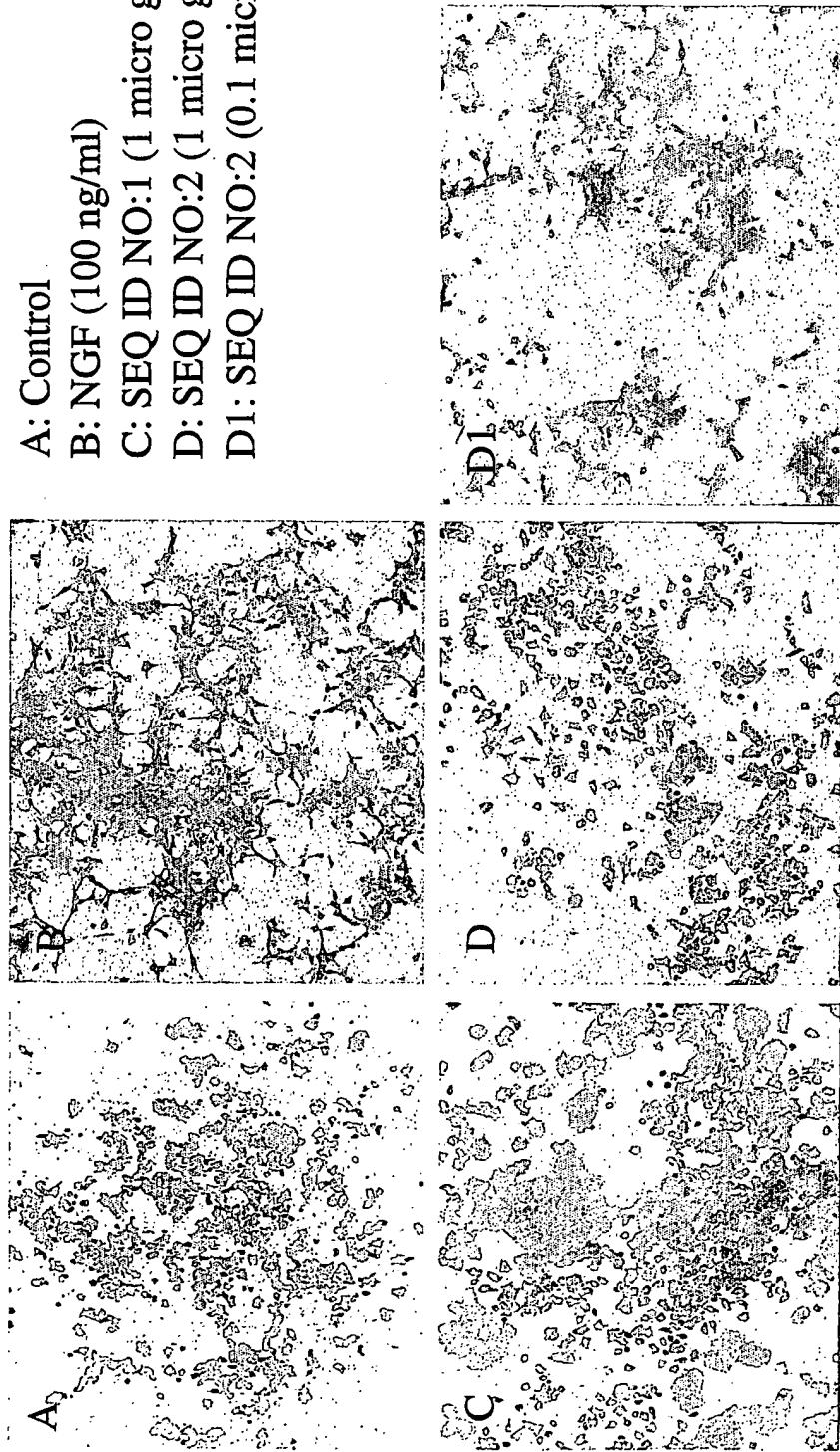


Fig. 1

## SEQUENCE LISTING

<110> THE UNIVERSITY OF TEXAS SYSTEM  
BOLDOGH, Istvan

<120> USE OF COLOSTRININ, CONSTITUENT PEPTIDES THEREOF, AND ANALOGS THEREOF  
TO PROMOTE NEURAL CELL DIFFERENTIATION

<130> 265.00240202

<140> Unassigned  
<141> 2000-08-17

<160> 34

<170> PatentIn Ver. 2.1

<210> 1  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 1  
Met Gln Pro Pro Pro Leu Pro  
1 5

<210> 2  
<211> 17  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 2  
Leu Gln Thr Pro Gln Pro Leu Leu Gln Val Met Met Glu Pro Gln Gly  
1 5 10 15

Asp

<210> 3  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 3  
Asp Gln Pro Pro Asp Val Glu Lys Pro Asp Leu Gln Pro Phe Gln Val  
1 5 10 15

Gln Ser

<210> 4  
<211> 12  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 4  
Leu Phe Phe Phe Leu Pro Val Val Asn Val Leu Pro  
1 5 10

<210> 5  
<211> 15  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 5  
Asp Leu Glu Met Pro Val Leu Pro Val Glu Pro Phe Pro Phe Val  
1 5 10 15

<210> 6  
<211> 11  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 6  
Met Pro Gln Asn Phe Tyr Lys Leu Pro Gln Met  
1 5 10

<210> 7  
<211> 15  
<212> PRT  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 7  
Val Leu Glu Met Lys Phe Pro Pro Pro Pro Gln Glu Thr Val Thr  
1 5 10 15

<210> 8  
<211> 15  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 8  
Leu Lys Pro Phe Pro Lys Leu Lys Val Glu Val Phe Pro Phe Pro  
1 5 10 15

<210> 9  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 9  
Val Val Met Glu Val  
1 5

<210> 10  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 10  
Ser Glu Gln Pro  
1

<210> 11  
<211> 3  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 11  
Asp Lys Glu  
1

<210> 12  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 12  
Phe Pro Pro Pro Lys  
1 5

<210> 13  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 13  
Asp Ser Gln Pro Pro Val  
1 5

<210> 14  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 14  
Asp Pro Pro Pro Pro Gln Ser  
1 5

<210> 15  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 15  
Ser Glu Glu Met Pro  
1 5

<210> 16  
<211> 7

<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 16  
Lys Tyr Lys Leu Gln Pro Glu  
1 5

<210> 17  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 17  
Val Leu Pro Pro Asn Val Gly  
1 5

<210> 18  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 18  
Val Tyr Pro Phe Thr Gly Pro Ile Pro Asn  
1 5 10

<210> 19  
<211> 9  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic  
peptide

<400> 19  
Ser Leu Pro Gln Asn Ile Leu Pro Leu  
1 5

<210> 20  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 20  
Thr Gln Thr Pro Val Val Val Pro Pro Phe  
1 5 10

<210> 21  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 21  
Leu Gln Pro Glu Ile Met Gly Val Pro Lys Val Lys Glu Thr Met Val  
1 5 10 15

Pro Lys

<210> 22  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 22  
His Lys Glu Met Pro Phe Pro Lys Tyr Pro Val Glu Pro Phe Thr Glu  
1 5 10 15

Ser Gln

<210> 23  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 23  
Ser Leu Thr Leu Thr Asp Val Glu Lys Leu His Leu Pro Leu Pro Leu  
1 5 10 15

Val Gln

<210> 24  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 24  
Ser Trp Met His Gln Pro Pro  
1 5

<210> 25  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 25  
Gln Pro Leu Pro Pro Thr Val Met Phe Pro  
1 5 10

<210> 26  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 26  
Pro Gln Ser Val Leu Ser  
1 5

<210> 27  
<211> 22  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 27  
Leu Ser Gln Pro Lys Val Leu Pro Val Pro Gln Lys Ala Val Pro Gln  
1 5 10 15

Arg Asp Met Pro Ile Gln  
20

<210> 28  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 28  
Ala Phe Leu Leu Tyr Gln Glu  
1 5

<210> 29  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 29  
Arg Gly Pro Phe Pro Ile Leu Val  
1 5

<210> 30  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 30  
Ala Thr Phe Asn Arg Tyr Gln Asp Asp His Gly Glu Glu Ile Leu Lys  
1 5 10 15  
Ser Leu

<210> 31  
<211> 9  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 31  
Val Glu Ser Tyr Val Pro Leu Phe Pro  
1 5

<210> 32  
<211> 13  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 32  
Phe Leu Leu Tyr Gln Glu Pro Val Leu Gly Pro Val Arg  
1 5 10

<210> 33  
<211> 3  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 33  
Leu Asn Phe  
1

<210> 34  
<211> 15  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic peptide

<400> 34  
Met His Gln Pro Pro Gln Pro Leu Pro Pro Thr Val Met Phe Pro  
1 5 10 15

## INTERNATIONAL SEARCH REPORT

Inte ia! Application No  
PCT/US 00/22777A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, MEDLINE, CHEM ABS Data, BIOSIS, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category <sup>o</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 14473 A (JANUSZ MARIN ; LISOWSKI JOZEF (PL); DUBOWSKA INGLOT ANNA (PL); HIRS) 9 April 1998 (1998-04-09) See especially page 2, lines 14-20; page 9, line 27 to page 10, line 12; claims 5-6 —	1-6, 9-11,14, 15
E	WO 01 12651 A (BOLDOGH ISTVAN ; UNIV TEXAS (US)) 22 February 2001 (2001-02-22) the whole document —	1-15
E	WO 00 75173 A (REGEN THERAPEUTICS PLC ; GEORGIADES JERZY A (US)) 14 December 2000 (2000-12-14) See especially page 5, lines 5-14; claims 12-13 — —/—	1-15

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the International search

12 June 2001

Date of mailing of the International search report

22/06/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Groenendijk, M

## INTERNATIONAL SEARCH REPORT

Inte  
al Application No  
PCT/US 00/22777

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 30686 A (SYNTEX INC) 16 November 1995 (1995-11-16) the whole document -----	1-15

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/US 00/22777

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
WO 9814473	A 09-04-1998	PL 316416	A	14-04-1998	
		AU 4565197	A	24-04-1998	
		BR 9712259	A	25-01-2000	
		CN 1238782	A	15-12-1999	
		EP 0932623	A	04-08-1999	
		GB 2352176	A,B	24-01-2001	
		GB 2333453	A,B	28-07-1999	
		HU 9904368	A	28-06-2000	
		JP 2001501929	T	13-02-2001	
		PL 332632	A	27-09-1999	
		TR 9901022	T	21-07-1999	
WO 0112651	A 22-02-2001	NONE			
WO 0075173	A 14-12-2000	AU	5093200	A	28-12-2000
WO 9530686	A 16-11-1995	US 5606031	A	25-02-1997	
		AU 697891	B	22-10-1998	
		AU 2432095	A	29-11-1995	
		CA 2189659	A	16-11-1995	
		EP 0772627	A	14-05-1997	
		JP 10502804	T	17-03-1998	